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Microbial Metabolomics: Fifty Shades of Metabolism

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THE IMPORTANCE OF METABOLISM IN INFECTIOUS DISEASE

Society owes no small debt to the academic study of infectious diseases. Throughout history, infectious diseases have remained a leading cause of deaths. Thankfully, sustained interest in microbes and the diseases they cause, dating to the 17th century germ theory of disease, has led to dramatic increases in life expectancy.

As with most areas of science, our understanding of infectious diseases has been defined by the technologies used to study it. Early culture-based approaches portrayed pathogens as microscopic aliens of predatory constitution and physiology. Nucleic acid-based approaches subsequently revised this view to reflect a competition of genomes for a given niche. This view was further advanced by the use of genetically defined animal models that revealed important differences between genes required for growth in a test tube and those required to cause disease in the host.

Interestingly, recent advances in analytical chemistry have begun to redefine infectious diseases yet again. Metabolism is a ubiquitous biochemical activity of all cells, depicted by most textbooks as a bulk housekeeping function of cells, relegated to the restocking of macromolecular precursors and generation of energy. However, growing reports of unexpected links between diverse physiologic and disease processes and specific metabolic enzymes and pathways have begun to challenge this view.

Metabolism is frequently portrayed as invariant in composition and structure. However, it remains a fact that metabolic networks fuel a diverse range of biochemical needs, according to the particular niches and selective pressures encountered by the cell or organism they serve. Accordingly, growing evidence has shown that metabolic networks can vary in composition and configuration, even when limited to a given set of enzymes. Metabolism is thus re-emerging as a chemically conserved, but multifunctional, mediator of cellular physiology.

From a functional perspective, metabolites are the final products of enzymes and enzyme networks and thus serve as the chemical arbiters of most, if not all, physiologic processes. Yet their identities and levels have largely eluded the analytical scope of traditional biochemical and genetic tools. It is in this context that metabolomics has emerged as the newest of the systems-level disciplines. Because metabolites reflect the integrated product of the genome, proteome, and environment, metabolomics represents a systems-level window into the biochemical state of a cell or organism, as reported by its complement of small molecule metabolites.

METABOLISM AS A DISCRIMINATORY LENS

Like other systems-level disciplines, metabolomics encodes information in both the pattern and composition of its component features. Metabolomic technologies have thus been used for both their discriminatory power and the biochemical information encoded by the metabolites they report.

In the context of discriminatory power, metabolomic approaches have been most directly applied to enable chemotaxonomic classification and/or identification of microbes. A clinically pertinent example of this is the emerging use of metabolomic profiles to noninvasively detect pathogenic bacteria in patient specimens by searching for signature volatile organic compounds (VOCs). Various mass spectrometric techniques are capable of detecting these chemicals, and researchers are currently building data sets that will establish pathogen-specific biomarkers to enable early disease diagnosis.[1]

In a more sophisticated approach, Layre and colleagues employed an untargeted comparison of the lipidomes of pathogenic and nonpathogenic mycobacterial species to discover a new family of molecules specific to virulent *Mycobacterium tuberculosis* (Mtb) strains.[2] This discovery was followed by detailed structural analysis and chemogenetic screen, which led to the identification of the biosynthetic pathway and genes responsible for its synthesis. Because the immunoregulatory properties of Mtb's lipids are a key mediator of its virulence, such knowledge is equally likely to expand our understanding of its pathogenicity. This study thus exemplifies the potential for discriminatory approaches to yield new biological insight.

In a more functionally oriented approach, this same discriminatory power has been used to annotate orphan enzyme activities lacking genomic assignments. By incubating recombinant proteins of unknown function with a highly concentrated extract of the homologous metabolome, it was specifically shown that potential substrates and products could be detected on the basis of time- and protein-dependent (and hence catalytic) changes in the composition of the metabolomic extract without a priori knowledge of its composition.[3] Similar comparative approaches have also been successfully applied using the native metabolomes of wild type and genetically defined deletion strains.[4] Like the case for nearly all sequenced microbial genomes, traditional biochemical and homology-based in silico approaches have failed to suggest a function for nearly 40% of microbial genes that presumably also include orphan enzyme activities for which no gene has been ascribed.[5] From a practical point of view, such genes and enzymes represent a particularly promising and biologically selective, but untapped, source of potential drug targets.

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METABOLISM AS A BIOLOGICAL LENS

From a more traditional biochemical perspective, metabolomics approaches have been applied to expand our understanding of the metabolic capabilities of pathogens. As stated previously, metabolic networks fuel the biochemical needs of pathogens, according to the specific niches and selective pressures they encounter. Growing evidence has thus revealed a remarkable degree of diversity. Studies of the closely related herpesviruses, cytomegalovirus and herpes simplex virus, for example, have revealed highly specific effects on host cell metabolism that are conserved across cell types but fuel distinct biochemical processes, whereas studies of influenza virus exhibited even more life cycle-specific changes essential for its pathogenesis.[6] Studies of the intracellular bacterial pathogen Mtb have similarly revealed an unusual ability to cocatabolize multiple carbon sources simultaneously in a functionally compartmentalized manner, perhaps reflecting an evolutionary adaptation to the nutrient-poor environment of its chief locale, the macrophage phagosome. Looking more deeply, such studies have recently begun to reveal new mechanistic links between known metabolic pathways and cellular functions, an important but frequently underappreciated goal of systems biology.[7]

Just as metabolism mediates pathogen biology, growing evidence is also establishing metabolism as a central determinant of host defense. For example, the activation of macrophages with different antigens can result in a phenotypic polarization into a cell whose role is either inflammatory and bactericidal (M1) or anti-inflammatory and antiparasitic (M2). Interestingly, recent work has shown that this reprogramming is fueled by a metabolic shift to provide molecules important for each function; M1 macrophages, for example, rely on aerobic glycolysis to promote arginine catabolism and bactericidal production of nitric oxide, whereas M2 macrophages rely on oxidative metabolism and arginase induction to affect wound repair.[8] In follow-up work, the construction of a metabolic map of macrophages upon lipopolysaccharide induction clearly implicated succinate accumulation in M1 reprogramming. The effect of this anapleurotic succinate production is stabilization of a protein (HIF-1α) that promotes interleukin-1β production, thus driving the inflammatory response.[9] To the extent that similar kinds of metabolic repurposing drive the phenotypes of other immune cells, it is possible that metabolomics may serve as a chemical lens into host defense.

Although still emerging, an area of particular interest will be to more fully define the roles of metabolism in the host–pathogen interface. Indeed, in a metabolomic analysis of an in vitro malaria infection model, Olszewski discovered arginine depletion as an important aspect of its proliferation in the red blood cell niche.[10]

METABOLISM AS A PHARMACOLOGIC LENS

From a more pragmatic perspective, metabolomic approaches have begun to introduce a new paradigm in anti-infectives research, termed intrabacterial pharmacology. Despite major advances in the fields of microbial pathogenesis and medicinal chemistry, only two new chemical classes of antibiotics have entered clinical medicine over the past 20 years. The causes of this shortfall are multifactorial. However, one major limitation of modern anti-infectives research has been the lack of direct biochemical readouts. Most antibiotics act on intracellular biochemical targets. Their activity is thus dependent on their ability to both reach and inhibit their specific biochemical targets within an intact bacterium. Yet current approaches in structure–activity relationship (SAR)-guided antibiotic development rest heavily on the use of indirect bacteriologic or genetic measures of compound activity. Although valuable, these tools and readouts have left critical ambiguities. For example, in developing a SAR around a given compound, it is difficult, if not impossible, to determine the extent to which the activity of a given compound is mediated by its ability to accumulate within a bacterial cell, its biochemical affinity for a specific target, and/or its biotransformation into one or more bioactive species. The ability of metabolomic technologies to chemically elucidate the intrabacterial pharmacokinetic fates and pharmacodynamic actions of compounds on the biochemical network of a given bacterium thus addresses a major unmet scientific need of rational anti-infectives development.[11]

Aside from development, metabolomic techniques have recently been applied toward the elucidation of mechanisms of action (MOA) for effective antibacterial compounds. This work follows the paradigm laid by earlier studies that used clustering to determine the function of uncharacterized genes in a compendium of expression profiles from *Saccharomyces* deletion mutants.[12] Complementary approaches based on metabolomic data sets are being developed to derive target identification for antibiotics effective against *Staphylococcus aureus*[13] and *Mycobacterium smegmatis*.[14] This approach has already borne fruit in the identification of the *S. aureus* pyruvate dehydrogenase complex as a putative target for a synthetic antimicrobial, triphenylbismuth dichloride.[15] It has also been used to reveal a novel inhibitory action by a drug with a known target, trimethoprim, which inhibits bacterial dihydrofolate reductase (DHFR). Using a metabolomic method to follow folate fluxes, Kwon and colleagues found that the accumulation of the substrate of DHFR (dihydrofolate) inhibits another enzyme of folate metabolism, thereby exacerbating the blockade of this essential pathway.[16] The capability to rapidly decipher MOA for attractive lead compounds promises to accelerate the drug development process by informing rational drug design approaches.

FUTURE PERSPECTIVES

Taking stock, it is intriguing to note the multiple forms of specificity encoded by intermediates of even the most widely conserved metabolic reactions and pathways. Metabolism is thus emerging as a complex chemical switchboard, rather than power plant, of cellular physiology. Given this specificity, it will be of particular interest to biochemically recast infectious diseases as a form of metabolic warfare, mediated by the dynamic ebb and flow of individual small molecules at the host–pathogen interface. To the extent that such efforts may reveal cell- or organism-specific metabolic codes, buried in the peaks of a chromatogram, society may finally begin to pay the academic study of infectious diseases forward.

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Notes

The authors declare no competing financial interest.

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